Bioved, **30**(1) : 19–24, 2019 http://biovedjournal.org/

Comparative study of the long term genotoxic effect of profenofos on swiss albino mice (*Mus musculus*)

Rina Kumari[1], Dharmshila Kumari[2], Alka Rani[2] and H.K. Chourasia[3]

1. P.G. Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur – 812 007
2. University Department of Zoology, T.M. Bhagalpur University, Bhagalpur – 812 007
3. University Department of Botany, T.M. Bhagalpur University, Bhagalpur – 812 007

Received November 2, 2018 and Accepted January 15, 2019

ABSTRACT : A large number of pesticides, insecticides, fertilizers etc. are widely used to protect the crops as well as stored gains but use of these chemicals may cause toxic effect in the body of consumers as well as non - target organisms. Profenofos is one of the organophosphate pesticides widely used on a variety of crops. It has adverse effect on many organs, immune system, haematological system etc. High doses of profenofos induced tissue vacuolization and haemorrhage while swelling of Bowmen's capsules and tubular degeneration in the kidney were well reported. Various reports are also available about the genotoxicity of profenofos (U.S. EPA, 1997; El-Bendary *et al.,* 2010; Bhinder and Chaudhary, 2013). The present study aims to assess the extent of long term genotoxic effect of profenofos at three different concentrations and durations. The extent of damages was assessed through the study of chromosomal aberrations in mice. When profenofos administered orally daily to Swiss albino mice for 30, 60 and 90 days at three different concentrations (lower, mild and higher), both structural and mitotic disruptive type of abnormalities were found in all the variants. However, mitotic disruptive changes were more pronounced than structural abnormalities in mice. The higher concentration of profenofos induced maximum abnormalities in comparison to mild and lower dose in 90 days, whereas minimum chromosomal abnormalities was observed in 30 and 60 days. This result shows that genotoxic effect of profenofos has been duration and dose dependent.

Key Words : Swiss Albino mice (*Mus musculus*), profenofos, genotoxicity, pesticide, chromosomal abnormalities.

Pesticides are chemical substances which has important role in modern agriculture. Despite cause environmental pollution, pesticide has serious health problems killing 250000 – 350000 people each year (Jeyaratnam, 1990; Gunnell, 2007). Organophosphate pesticide which has been classified as Toxicity class II by WHO. Pesticides has great potential in protecting the crop from seedling to harvest stage and even during storage of grains. Whenever pesticides are sprayed over the plant parts (root, stem, leaves, flowers and fruits) they are absorbed effectively from the external surfaces (Matsumura, 1970). The pesticide residues most often persist for longer period of time inside the plant (Gupta *et al.*, 1977; Yadav and Srivastava, 1982). When non-targeted organisms such as man, birds, animals are consume the leaves, fruits or grains of the plant, the pesticide residues enter into their body through the diet (Grlic, 1988). Like other organophosphate, the profenofos mechanism of action is via the inhibition of acetylcholinesterase enzyme which catalyse the breakdown of acetylcholine that function as neuro-transmitters. Hence, profenofos inhibit the function of neurotransmitters and prevent the neurotransmission process. Therefore, profenofos has first effect on the nervous system in animals as well as human (Gupta *et al.*, 2001). The rapidity of inhibition of acetylcholinesterase enzyme by profenofos is depends on the dose of profenofos (Mishra *et al.,* 2015). Some other organs like brain, kidney, liver, muscles, pancreas, immune system, reproductive system and urinary system are also adversely affected by profenofos (Akhgari *et al.,* 2003; Gupta, 2006; Mansour *et al.*, 2009; Shrutilekha *et al.*, 2014). Profenofos pesticides have been widely studied for their ability to induce damage to DNA *in vivo* and *in vitro* systems and have also genotoxic, alkylating and clastogenic properties (Mehta *et al.,* 2008; Ojha and Srivastava, 2014). It is reported to cause chromosomal aberration, micronuclei formation and sister chromatid exchange in the bone marrow of the mice (Das *et al.*, 2006). The effect of various concentrations of the pesticides on the chromosomes of mitotically dividing bone morrow cells were studied to search out a threshold dose level.

The dose and time dependant effect of organophosphate pesticide have also been studied in mammals. The genotoxicity of pesticide has been found to be both duration and dose dependent (Mitra and VG, 2016; Jayashree *et al.*, 1994). Hence, the present study is aimed to study the long term genotoxic effect of profenofos in mice at three different concentrations (low, mild and high) and

Table-1 : Summary of the treatment protocol.

S.No.	Exprimental variants	Symbol	Dose
1.	Control	C	No P1,P2,P3
2.	Profenofos (lower dose)	P_1	0.5 mg/ml
3.	Profenofos (mild dose)	p_2	1 mg/ml
4.	Profenofos (higher dose)	p_3	2 mg/ml

durations (30, 60, 90 days).

Materials and Methods

Chemicals

Commercially available Profenofos, [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] (50% E.C, trade name: "Carina", PI Industries Ltd.) was purchased from the local market, Bhagalpur (Bihar).

Animals

Four to five week old healthy Swiss Albino mice (*Mus musculus*) were obtained from the laboratory inbred stock and maintained in the animal house of the P.G. Department of Zoology, T.M.B.U., Bhagalpur and kept under the standard laboratory condition. The animals were fed on food grains and tap water. Treatment and protocols employed in this study were done after proper approval of the Institutional Head and Departmental Research Committee.

Treatment

Both sexes of mice were put into four groups and subjected to various treatments at three different concentrations (Table-1). The first group was taken as control, second group treated with lower dose (0.5 mg/ml), third group with mild dose (1 mg/ml) and fourth one with higher dose (2 mg/ml). The lower dose was the half of the mild dose and higher dose was double of the mild dose. The lethal dose of profenofos was 25 mg/kg/b.w. (Kumar *et al.,* 2011).

Slide preparation

After 30, 60 and 90 days, of treatment animals were sacrificed by cervical dislocation and slides of mitotic metaphase chromosome were prepared from bone marrow cells of the mice by colchicine-hypotonic-acetoalcohal-flame-drying-giemsa staining technique. (Preston *et al.,* 1987).

Slide screening

300 well spread mitotic metaphase plates were screened under the microscope for the study of structural and mitotic disruptive abnormalities in each variants. Student t-test was applied for the data calculation.

Results and Discussion

Both structural and mitotic disruptive types of abnormalities were found in all the variants at different duration and different concentration of profenofos. The percentage of total chromosomal abnormalities for 30, 60 and 90 days at different concentration (lower, mild and higher) were significantly higher than control (Table-2). In 30 days of treatment duration, the percentage of total abnormalities was found to be 12.3 $\pm$ 1.89 at lower concentration, 15.6 $\pm$ 2.09 at mild concentration and 24 $\pm$2.59 at higher concentration, which is significantly higher than control 5.3 $\pm$ 1.29.

In 60 days of treatment, the percentage of total abnormalities was found to be 30 $\pm$2.64 at lower concentration, 39.6 $\pm$ 2.82 at mild concentration and 44 $\pm$ 2.86 at higher concentration which is also significantly higher than control and 30 days treatment of different concentration of profenofos.

In 90 days of treatment, the percentage of total abnormalities was found to be 34 $\pm$ 2.73 at lower concentration, 42 $\pm$ 2.84 at mild concentration and 52.6 $\pm$2.88 at higher concentration than control 5.3 $\pm$1.29 as well as 30 and 60 days treatment of different concentration of profenofos.

Among both types of abnormalities, mitosis disruptive was more frequency than structural abnormalities in all variants. The acentric fragments, breaks, metacentric chromosome, gap and ring were more common in structural type abnormalities and polyploidy, hypoploidy, stickiness and clumping were found in mitosis disruptive type of abnormality. Therefore, from the aforesaid study it can be concluded that exposure of profenofos was dose and duration dependent in mice.

The toxicity of organophosphate depends on their chemical structure, metabolism in target organism, concentration, mode of application etc. in animals as well as human (Grlic *et al*., 1988). It was also reported that profenofos induced free radical and oxidative tissue damage in animal and human (Bagchi *et al.,* 1995). In

Table-2 : Incidence of metaphase chromosome abnormality in mitotic cell of mice treated with three concentrations of profenofos pesticide for 30, 60 and 90 days.

Sl.	Symbol	Dose (mg/kg)	Time in Days	Structural Abnormalities			Numerical Abnormalities			Total Abnormalities		
				No.	%	± S.E	No	%	± S.E	No.	%	±S.E
1.	C	—	—	6	2	± 0.80	10	3.3	± 1.02	16	5.3	±1.29
2	P_1	0.5	30	16	5.3	±1.29	21	7	±1.47	37	12.3	±1.89*
3	P_2	1	30	20	6.6	±1.43	27	9	±1.65*	47	15.6	±2.09*
4	P_3	2	30	33	11	±1.80*	39	13	±1.94*	72	24	±2.59*
5	P_1	0.5	60	32	10.6	±1.77*	58	19.3	±2.27*	90	30	± 2.64 *
6	P_2	1	60	47	15.6	±2.09*	72	24	±2.46*	119	39.6	±2.82*
7	P_3	2	60	59	19.6	±2.29*	73	24.3	±2.47 *	132	44	±2.86*
8	P_1	0.5	90	40	13.3	±1.9**	62	20.6	±2.33**	102	34	±2.73**
9	P_2	1	90	52	17.3	±2.18**	74	24.6	±2.48**	126	42	±2.84**
10	P_3	2	90	80	26.6	± 6.50**	78	26	±2.53**	158	52.6	±2.88**

*Indicate significant difference with respect to control.

**Indicate significant difference with respect to control along with 30 days duration.

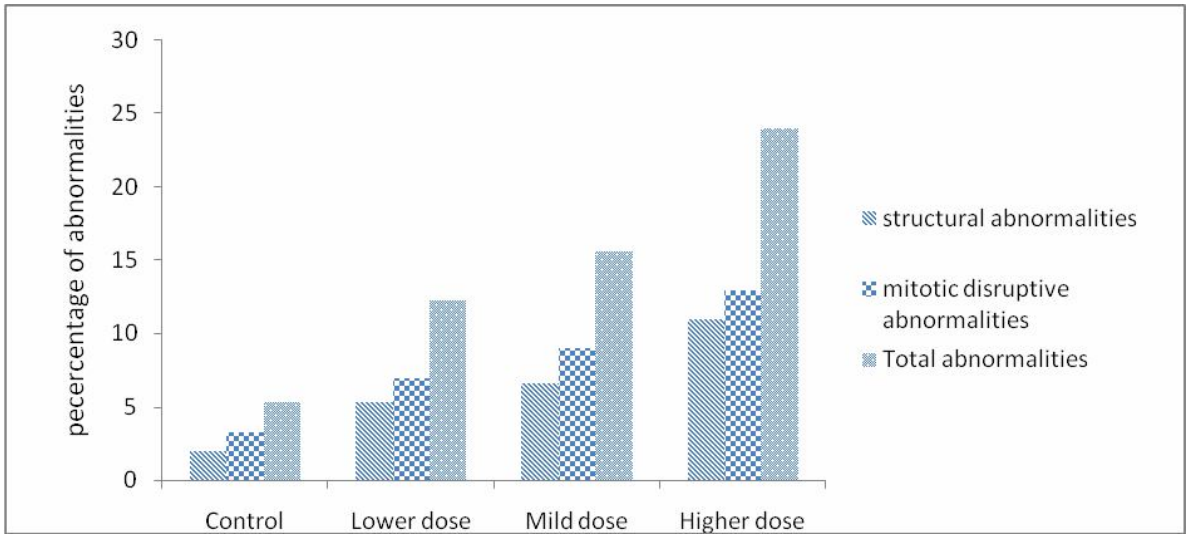

Fig-1 : Graphical representation of chromosomal abnormalities for 30 days.

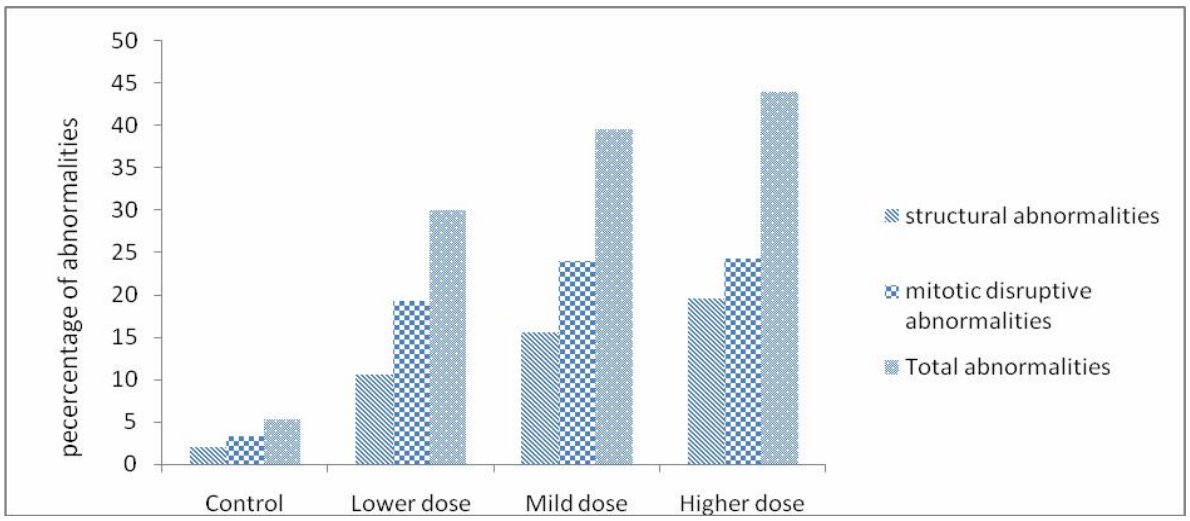

Fig.-2 : Graphical representation of chromosomal abnormalities for 60 days.

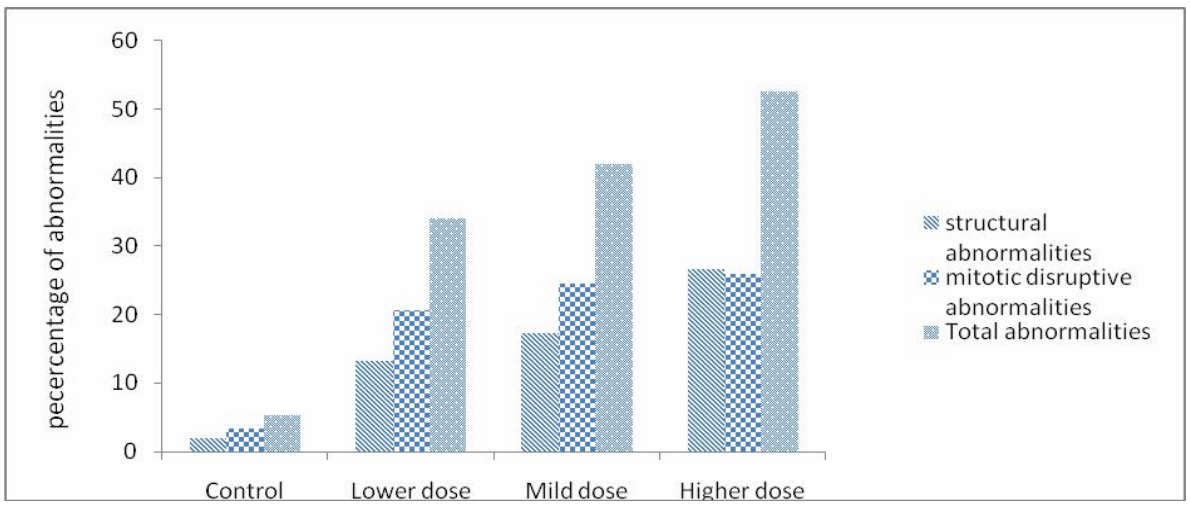

Fig.-3 : Graphical representation of chromosomal abnormalities for 90 days.

previous study, it has been found that some other organophosphate pesticide has also genotoxic property such as methyl parathion, malathion, hinosan and fenthion (Ojha *et al.,* 2013; Wu *et al.,* 2011; Jayashree *et al.,* 1994). A growing number of studies recently have been investigated the genotoxic effect of organophosphate pesticide on animal model *in vitro* (Jayashree *et al.,* 1994; El-Khatib and Shalaby, 2001; Hammam and Mottaleb, 2007; El-Bendary *et al.,* 2010).

In present investigation, we found that total chromosomal abnormalities of higher dose of profenofos in 90 days has maximum level of genotoxicity compare to 30 and 60 days, this finding is similar with observation of Bhunya and Behera (1984) who observed a dose dependent frequency of chromosomal abnormalities induced by pesticides. It is also found that the frequency of chromosomal abnormalities were less increase during the 90 days duration at all three different concentration of profenofos in comparison to 60 days duration of three different concentration. The percentage of the total chromosomal abnormalities were increased with increase in concentration and duration. Therefore, this study was found to be dose and duration dependent.

Acknowledgment

The authors are thankful to Professor M.C. Verma, Head, University Department of Zoology, T.M. Bhagalpur University, Bhagalpur for providing laboratory facility on animal system.

References

Akhgari, M.; Abdollahi, M.; Kebryaeezadeh, A.; Hosseini, R. and Sabzevari, O., 2003. Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum ExpToxicol.*, **22 :** 205-211.

Bagchi, D.; Bagchi, M.; Hassoum, E.A. and Stohs, S.J., 1995. *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage of selected insecticides. *Toxicol.*, **104**(1-3) **:** 129-140.

Bhinder, P. and Chaudhary, A., 2013. Genotoxicity of acephate and profenofos assessed by PCR assay. *Int. J. Pharma. Res. and Bio-sci.*, **2**(4) : 280-290.

Bhunya, S.P. and Behera, J., 1984. Clastogenicity of a fungicide, Edifenphos (Hinosan) in the bone marrow cells of mice *in vivo*, *Cytologia,* **49 :** 833-839.

Das, G.P.; Shaik, A.P. and Jamil, K., 2006. Cytotoxicity and Genotoxicity induced by the pesticide profenofos on cultured human peripheral blood lymphocytes. *Drug and Chemtoxicol.*, **29:** 313-322.

El-Bendary, H.M.; Negam, S.E.; Saleh, A.A.; Kady, M.M. and Hosam Eldeen, F.A., 2010. Genotoxic and probable mutagenic effects of some pesticides on mice bone marrow cells. *J. Plant Protection and Pathology, Mansoura Univ.,* **1**(9) : 681-696.

El-Khatib, E.N. and Shalaby, R.H., 2001. Genotoxic effects of two pesticides and their mixture: In-vivo chromosomal aberration and micronucleus assay. *J. Union. Arab.Bilo.*, **16**(A) : 355-380.

Gunnell, D.; Eddleston, M.; Phillips, M.R. and Konradsen, F., 2007. The global distribution of fatal pesticide self-poisoning: systematic review. *BMC Public Health,* **7:** 357.

Grlic, L., 1988. Mali kemijskileksikonNapried. *Zagreb.*

Gupta, H.K.L.; Kushwaha, K.S. and Srivastava, B.P., 1977. Uptake of Aldrin residues in root crop from treated soil. Symp. on insect and environ. *Univ. of Delhi.* 38.

Gupta, R.C., 2006. Toxicology of organophosphates and carbamate compounds. *Elsevier Academic Press* : 5-24.

Gupta, S.; Stravitz, R.T.; Dent, P. and Hylemon, P.B., 2001. Down-regulation of cholesterol 7alpha-hydroxylase (CYP7A1) gene expression by bile acids in primary rat hepatocytes is mediated by the c-Jun N-terminal kinase pathway. *J Biol Chem.,* **276**(19): 15816-15822.

Hammam, M.F and Mottaleb, M.A., 2007. Studies of the genotoxic and Histopathological effects of the organo-phosphorous insecticide profenofos on white rats. *The Egyptian Journal of Hospital Medicine,* **29 :** 685-706.

Jayashree, I.V.; Vijayalaxmi, K.K. and Rahiman, M.A., 1994. The genotoxicity of Hinosan, an organophosphorus pesticide in the *in vivo* mouse. *Mut. Res,* **322 :** 77-85.

Jeyaratnam, J., 1990. Acute pesticide poisoning a major global health problem. *World Health Stat. Q.,* **43:** 139-144.

Mansour, M.K.; El-Kashoury, A.A.I.; Rashed, M.A. and Koretem, K.M., 2009. Oxidative and biochemical alterations induced by profenofos insecticide in rats. *Nature and Science,* **7**(2) : 1545-0740.

Mehta, A.; Verma, R.S. and Srivastava, N., 2008. Chlorpyrifos-induced DNA damage in rat liver and brain. *Environ. Mol. Mutagen,* **49 :** 426-433.

Mishra, V.; Sharma, S.; Khatri, S. and Srivastava, N., 2015. Evaluation of genotoxicity of monocrotophos and quinalphos in rats and protective effects of melatonin. *Integr. Pharm. Toxicol. Gentoxicol.*, **1**(1) : 33-42.

Mitra, D. and VG, A., 2016. Genotoxic effect of pesticides on human leukocyte culture: A review. *Asian J. of Pharm. and Clin. Res.,* **9**(5) : 29-33.

Ojha, A. and Srivastava, N., 2014. *In vitro* studies on organophosphate pesticides induced oxidative DNA damage in rat lymphocytes. *Mutat Res Genet Toxicol*

Environ. Mutagen., **761:** 10-17.

Ojha, A.; Yaduvanshi, S.K.; Pant, S.C.; Lomash, V. and Srivastava, N., 2013. Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues. *Environ Toxicol.*, **28 :** 543-552.

Preston, R.J.; Dean, B.J.; Galleway, S.; Holden, H.; Mcfree, A.F. and Shelby, M., 1987. Mammalian in vivo cytogenetic assay analysis of chromosome aberration in bone marrow cells.*Mut Res.*, **189 :** 157-165.

Shrutilekha; Singh, J.K.; Kumari, S. and Verma, R.K., 2014. Impact of blumealacera on profenofos exposed kidney of wistar rat (*Rattus novergius*). *Int. J. of Basic and Appli. Sci. Res.*, **1**(2) : 100-104.

U.S. EPA, 1997. Department of pesticides regulation, medical toxicology branch: summary of toxicology data profenofos. *Washington, DC:U.S. EPA*.1-10.

Wu, J.C.; Hseu, Y.C.; Tsai, J.S.; Chen, L.C. and Chye, S.M., 2011. Fenthion and terbufos induce DNA damage, the expression of tumor-related genes, and apoptosis in HEPG2 cells. *Environ Mol Mutagen.*, **52:** 529-537.

Yadav, P.R. and Srivastava, B.P., 1982. Presence of BHC in soils, its uptake in maize and pearl millet crops raised on soils treated in previous season. *Proc. Nat. Acad. Sci. India*, **52**(B) **:** 373-383.